

EXHIBIT A134

Retrograde migration of glove powder in the human female genital tract

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BACKGROUND: This study in humans was undertaken to evaluate earlier results from animal research showing a retrograde migration of glove powder from the vagina into the intra-abdominal cavity. **METHODS:** One study group was gynaecologically examined with powdered gloves the day before an abdominal hysterectomy and another group 4 days pre-operatively. There were two control groups similarly examined with powder-free gloves. Cell smears were taken from the peritoneal fluid and during the operation further smears were taken from the Fallopian tubes, uterine cavity and cervical canal. **RESULTS:** Statistically significant differences were found for large starch particles at all locations between the study and control groups examined 1 day pre-operatively. Considering small starch particles, there were significant differences in cervix ($P < 0.001$), uterus ($P < 0.01$) and the Fallopian tubes ($P < 0.01$). The combined results also show significant differences between both large and small starch particles in cervix, uterus and the Fallopian tubes. There were also differences between the study and control groups examined 4 days pre-operatively, but these were not statistically significant except for small and large starch particles in uterus ($P < 0.01$, $P < 0.05$) and cervix ($P < 0.05$, $P < 0.05$). **CONCLUSIONS:** This study has pointed out a retrograde migration of starch also in humans after a gynaecological examination with powdered gloves. Consequently, powder or any other potentially harmful substance that can migrate from the vagina should be avoided.

Key words: female/gloves/retrograde migration/starch particles/vaginal examination

Introduction

Earlier case reports suggest that intra-abdominal granulomas or adhesions due to starch particles were caused by starch powder used on gloves during vaginal examination. An initial indication of retrograde flow through the Fallopian tubes was the finding of intraperitoneal starch granulomas (Paine and Smith, 1957). Later the first case of starch peritonitis in a patient without previous surgery was reported (Saxen *et al.*, 1963). A recent investigation detected talcum particles on the ovaries in women who had used perineal talc applications (Heller *et al.*, 1996). In contrast, tubal ligation prevents the access of mediators that reach the peritoneal cavity through the Fallopian tubes (Ylikorkala, 2001).

Powder-free gloves have been available for 20 years, but starch-powdered gloves are still available and in use (Sjösten *et al.*, 1999).

It is well documented that starch-powdered gloves are not appropriate for abdominal surgery (Ellis, 1990; Holmdahl *et al.*, 1994), and intraperitoneally, starch particles can initiate inflammatory reaction and the formation of adhesions (Edelstam *et al.*, 1992; diZerega, 1994), although the mechanism by which starch increases the propensity of tissues to

form adhesions is not known. Reduced peritoneal fibrinolysis and activation of leukocytes by particulate starch granules have been suggested as possible mechanisms. Activated leukocytes, particularly macrophages, produce supernormal amounts of oxygen-free radicals, prostaglandin E₂, thromboxane B₂ and various cytokines (Osman and Jensen, 1999). Starch particles also increase the eicosanoid production which may contribute to the inflammatory or immune reactions and development of adhesions (Chegini and Rong, 1999). If already injured mesotelial surface of the peritoneum is exposed to starch, more dense adhesions are created compared to the effect of peritoneal trauma or starch separately. Application of glove powder on minimally or severely traumatized peritoneum facilitates tumour cell adhesion and growth alone (van den Tol *et al.*, 2001). Histological re-evaluation after tubal reconstructive surgery due to peritubal or peri-ovarian adhesions has shown residual starch from powdered gloves (Yaffe *et al.*, 1980).

A causal connection has been shown between operative tissue damage, intra-abdominal ischaemia, infections, reactions to foreign materials such as sutures, particles of gauze, glove dusting powder and post-operative adhesions

A.C.E.Sjösten, H.Ellis and G.A.B. Edelstam

(Mylärniemi, 1967; Holmdahl *et al.*, 1996). One of the proven causes of post-operative intestinal adhesions is microscopic foreign bodies which are present in up to 93% of adhesions (Duron *et al.*, 1997). After open abdominal or pelvic surgery, a third of the patients are readmitted at least twice during the subsequent 10 years for a disorder directly or possibly related to adhesions (Ellis *et al.*, 1999).

Our previous investigation in a rabbit model indicated a retrograde migration of glove powder from the vagina into the intra-abdominal cavity (Edelstam *et al.*, 1997). The amount that reaches the peritoneum is sufficient to significantly increase formation of post-operative adhesions after a standardized trauma (Sjösten *et al.*, 2000).

Therefore, this subsequent study in humans was done to investigate whether starch particles from powdered gloves also in humans might gain access to the abdominal cavity through the vagina after a gynaecological examination with powdered gloves.

Materials and methods

Patients

The participants in the study were divided into four different groups. Informed consent was obtained from all participants. All had a routine gynaecological examination before an elective laparotomy for total or subtotal hysterectomy due to fibroids or menometrorrhagia. Group I: examined 1 day pre-operatively with (i) powdered gloves (Gammex® Ansell GmbH, Germany; $n = 17$, mean age 51 years) or (ii) powder-free gloves (Biogel® Regent Medical, SLL) ($n = 15$, mean age 51 years). Group II: examined 4 days pre-operatively with (i) powdered gloves ($n = 12$, mean age 53 years) or (ii) powder-free gloves ($n = 14$, mean age 52 years). Patients with cancer of the uterus were excluded as well as women with ongoing menstrual bleeding. The pre-menopausal women were examined regardless of the follicular or luteal phase of the menstrual cycle. A third of all women in the study were post-menopausal. Any medication that might have influenced the tubal patency had not been taken except in the case of three patients who had an asthmatic disease and needed to take terbutaline occasionally. The medication was not taken during the investigations. There were no other significant differences for patient characteristics. Sexual activity, cyclic changes or hormonal effect were not considered in this study.

Surgical procedure

An abdominal subtotal or total hysterectomy was undertaken with the operating team and the nurse who set up the instrument tray wearing powder-free gloves. Immediately the abdominal cavity was opened, peritoneal fluid was collected and cell smears were then taken from the peritoneal fluid. From the fimbriae of the Fallopian tubes, additional cell smears were taken per-operatively and when the uterus had been removed, i.e. post-operatively from the uterine cavity and the cervical canal. For making the smears sterile, forceps or peans were used. Smears from the fimbriae of the Fallopian tubes were omitted if they were not removed during the hysterectomy.

Cell smears

The cell smears were quantitatively standardized on $\sim 1 \text{ cm}^2$ of one-half of a glass slide with the other blank side serving as control for contamination with air-borne starch particles. All the slides were stained with May-Grünwald Giemsa by a biochemical assistant wearing powder-free gloves in a laboratory where only powder-free

Table I. Small and large starch particles on day 1 after examination with powdered (Ia) and powder-free (Ib) gloves respectively

		No. of patients	Total no. of particles	Median	Range	Mean	P
Cervix							
Small	Ia	17	70	1	14	4.1	< 0.001
	Ib	15	0	0	0	0	
Large	Ia	17	46	0	24	2.7	< 0.01
	Ib	15	1	0	1	0.01	
Uterus							
Small	Ia	17	104	2	48	6.1	< 0.01
	Ib	15	0	0	0	0	
Large	Ia	17	22	0	10	1.3	< 0.01
	Ib	15	1	0	1	0	
Fallopian tubes							
Small	Ia	12	34	1.5	16	2.8	< 0.01
	Ib	13	0	0	0	0	
Large	Ia	12	18	0	10	1.5	< 0.05
	Ib	13	0	0	0	0	
Peritoneal fluid							
Small	Ia	13	13	1	4	1.0	NS
	Ib	13	3	0	3	0.2	
Large	Ia	13	12	0	6	0.9	< 0.05
	Ib	13	0	0	0	0	

NS = not significant.

gloves were used. The slides were coded and analysed by two independent investigators with a Zeiss 4/76 microscope using polarized light at magnification $\times 250$. The starch particles were counted in a standardized procedure for all slides. The numbers on the blank side (i.e. contamination) were subtracted from that in the smears so that the number of starch particles on each slide represent the net number without contaminating particles. Since there are differences in the size of starch particles they were divided into two sizes: (i) smaller than a leukocyte and (ii) larger than a leukocyte. Leukocytes for comparison in size were always present in the smears. The study was approved by the local ethics committee.

Statistics

Non-parametric Mann-Whitney *U*-tests and Fisher's exact test were used and values are given as SEM for the group. Differences were considered significant at the $P < 0.001$, $P < 0.01$ and $P < 0.05$ levels. All statistical tests were computerized and carried out with statistics programs (Statistica™; Statsoft, USA).

Results

Group I: examined 1 day pre-operatively with (i) powdered gloves ($n = 17$) and (ii) powder-free gloves ($n = 15$)

Starch particles were found in the cell smears with more particles found on the slides from the patients examined with powdered gloves. The differences were significant at all locations in the genital tract for small particles (cervix $P < 0.001$, uterus and Fallopian tubes $P < 0.01$) and large particles (cervix and uterus $P < 0.01$ and Fallopian tubes $P < 0.05$) but only for large particles in the peritoneal fluid ($P < 0.05$). However, in two patients examined with powdered gloves, no particles were found. On the contrary, in three patients examined with powder-free gloves, a few particles were found (Table I and Figure 1).

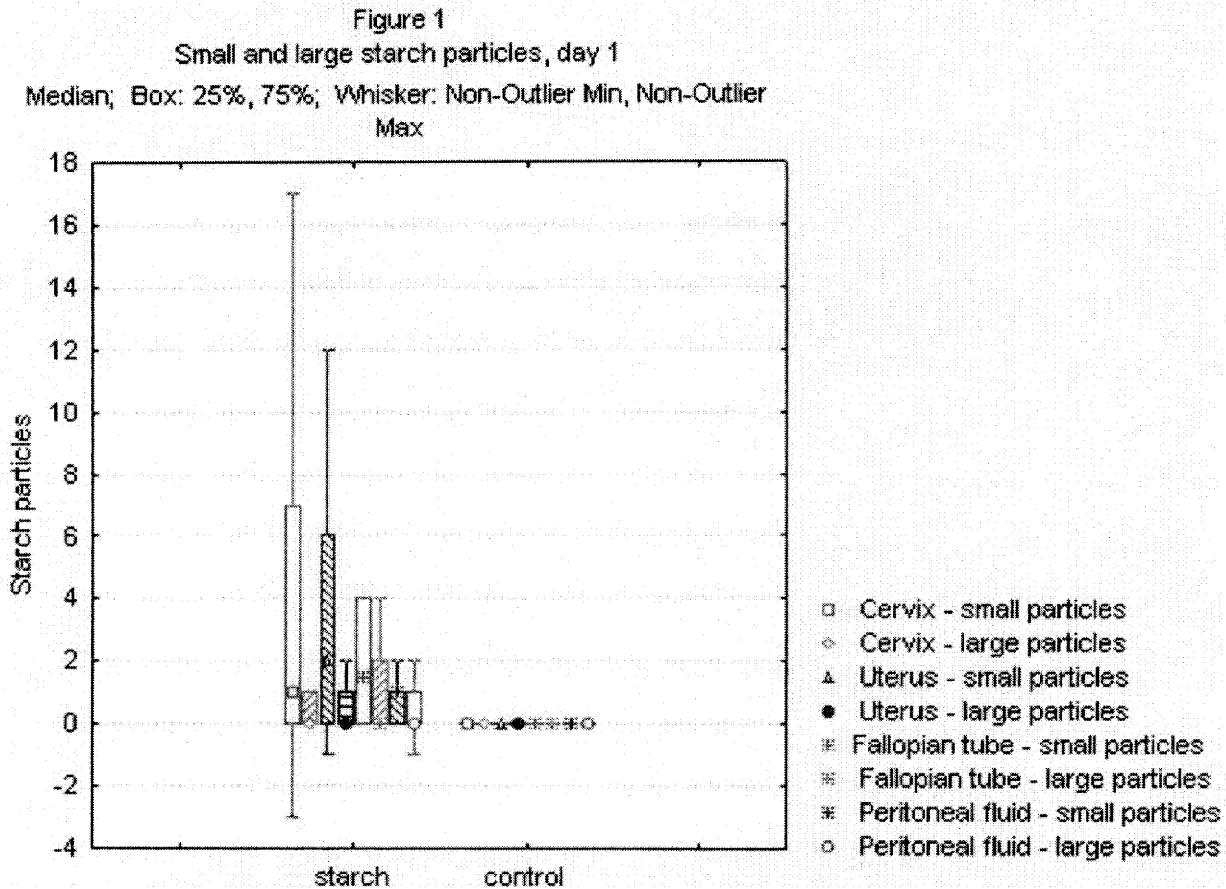


Figure 1. Median and range value for the retrograde transportation of small and large starch particles respectively, in different locations 1 day after a gynaecological examination with or without powdered gloves. The negative range value in the starch group for cervix, uterus and peritoneal fluid are due to contamination with airborne starch particles.

Group II: examined 4 days pre-operatively with (i) powdered gloves ($n = 12$) and (ii) powder-free gloves ($n = 14$)

There were significantly more small starch particles as well as large particles (cervix and uterus $P < 0.05$) after examination with powdered gloves. The differences were the same for small particles but less significant for large particles (uterus $P < 0.05$). The differences were non-significant in the Fallopian tubes and the peritoneal fluid (Table II and Figure 2).

Discussion

Medical gloves for use in surgery were introduced in 1896. Since then, several additives have been tried to facilitate manufacturing and to reduce the hazards associated with glove use (Ellis, 1990). Rubber and glove lubricants are the two main components in modern gloves. Starch powder as a glove lubricant can lead to complications such as granulomatous peritonitis (Giercksky *et al.*, 1994), adhesion formation (van den Tol *et al.*, 2001) and potentiation of infection (Renz and Gemsa, 1997), with subsequent intestinal obstruction, infertility and chronic pelvic pain.

Table II. Numbers of small and large starch particles after examination with powdered (IIa) and powder-free (IIb) gloves respectively, day 4

		No. of patients	Total no. of particles	Median	Range	Mean	P
Cervix							
Small	IIa	12	26	1	2	2.1	< 0.05
	IIb	14	0	0	0	0	
Large	IIa	12	9	0	3	0.8	< 0.05
	IIb	14	0	0	0	0	
Uterus							
Small	IIa	12	21	3	20	1.8	< 0.01
	IIb	14	2	0	0	0.1	
Large	IIa	12	7	0	3	0.6	< 0.05
	IIb	14	0	0	0	0	
Fallopian tubes							
Small	IIa	11	16	1	5	1.4	NS
	IIb	14	4	0	1	0.2	
Large	IIa	11	2	0	1	0.2	NS
	IIb	14	0	0	0	0	
Peritoneal fluid							
Small	IIa	9	14	1	5	1.6	NS
	IIb	11	3	0	1	0.3	
Large	IIa	9	2	0	1	0.2	NS
	IIb	11	0	0	0	0	

NS = not significant.

A.C.E.Sjösten, H.Ellis and G.A.B.Edelstam

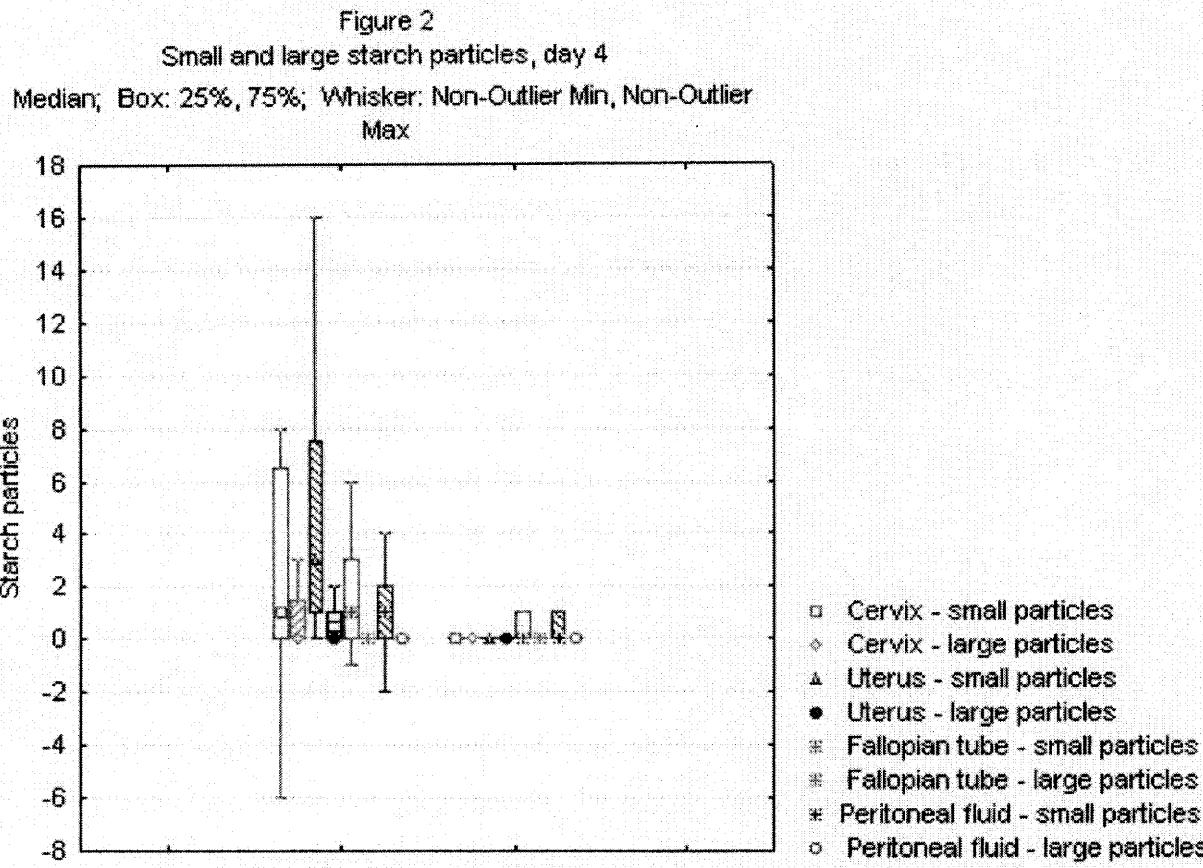


Figure 2. Median and range value for the retrograde transportation of small and large starch particles respectively, in different locations 4 days after a gynaecological examination with or without powdered gloves. The negative range value in the starch group for cervix, Fallopian tube and peritoneal fluid are due to contamination with airborne starch particles.

The possibility of retrograde migration of starch particles in the female genital tract into the intraperitoneal cavity has been suspected for several decades (Saxen *et al.*, 1963). The present study in humans has attempted to investigate whether previous results from animal research—that starch particles can migrate from the vagina into the abdominal cavity (Edelstam *et al.*, 1997)—reflects the case in humans. This study indicates such a retrograde migration of starch particles after gynaecological examination with powdered gloves. There were statistically significant differences between study and control groups in cervix, uterus and Fallopian tubes on the first day after vaginal examination with powdered gloves compared to powder-free examination. The low number of starch particles in the cell smear of the peritoneal fluid may reflect differences in the total amount of fluid and that it might have been better to collect all the fluid and after centrifugation prepare cell smears. However, with the present approach a significant difference between pre-operative examination with powdered and powder-free was demonstrated. The lower number of particles on the fourth day might indicate that absorption of starch particles had started, or that the particles had adhered to the peritoneum. In previous animal studies, most particles were found on the third day after

deposition in the vagina (Edelstam *et al.*, 1997). The numbers found in the controls indicate that the presence of starch particles in the peritoneal cavity is in accordance with reported persistence for up to 18 months (Ellis, 1971). Our present patients have been examined in that time before the referral for hysterectomy.

A considerable number of gynaecologists wears starch-powdered gloves (Sjösten *et al.*, 1999), despite evidence of starch-induced complications. The starch particles can migrate not only from the vagina into the cervical canal and the uterine cavity but also through the Fallopian tubes into the peritoneal fluid. Women exposed to intra-abdominal surgical trauma 1–4 days after a gynaecological examination with powdered gloves may be at increased risk of intra-abdominal adhesions. But even without a surgical procedure there is a risk of intra-abdominal or peri-tubal adhesions due to the examination with powdered gloves (Osser *et al.*, 1989). Ongoing subclinical PID can cause infective tissue damage. An extensive study by Myllärniemi (1967) showed that talc, starch powder and lint in the abdominal cavity tended to accumulate in the traumatized areas of the peritoneum so that the foreign material contaminating the peritoneal tissues could act together with other

Retrograde vaginal migration of glove powder in humans

traumatizing conditions, possibly preventing the resorption of fibrinous adhesions. This corresponds to our previous finding in the rabbit model that starch particles deposited in the vagina can migrate in a retrograde direction from the vagina into the abdominal cavity and, combined with an intra-abdominal trauma, generate dense adhesions (Sjösten *et al.*, 2000). Since there are indications towards retrograde migration of powder, it must not be used regardless of cyclic variations or sexual activity.

In conclusion, our results show that starch particles can migrate from the vagina into the cervical canal, the uterine cavity and through the Fallopian tubes up to 4 days after a gynaecological examination with powdered gloves. Glove powder contributes to adverse intra-abdominal reactions, which include adhesion formation and adhesion-related complications such as chronic pelvic pain and bowel obstruction. Tubal and pelvic adhesions are a major cause of female infertility. Since evidence suggests that a retrograde migration could be a general mechanism, our recommendation is that we should be critical of harmful substances, e.g. glove powder, that could migrate from the vagina to abdominal cavity.

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